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Production of extracellular matrix by glomerular epithelial cells is regulated by transforming growth factor-β1

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Production of extracellular matrix by glomerular epithelial cells is regulated by transforming growth factor-β1. Transforming growth factor-β (TGF-β) has widespread effects on extracellular matrix production by many cultured cell lines and appears to play a role in the pathological accumulation of extracellular matrix that accompanies inflammatory and fibrotic diseases such as glomerulonephritis. Earlier experiments have shown that mesangial cells respond to TGF-β1 with a marked increase in the production of two chondroitin/dermatan sulfate proteoglycans, decorin and biglycan, but their production of other matrix components elevated in glomerulonephritis is not substantially affected by TGF-β1. Since the glomerular epithelial cells are also thought to contribute to matrix production in the glomerulus, we examined the ability of these cells to produce some of the nonproteoglycan matrix components in response to TGF-β1. Exposure of glomerular epithelial cells to TGF-β1 increased the production of fibronectin and type IV collagen, in addition to biglycan. Enhancement of the cell layer accumulation of laminin was also observed. These results show that TGF-β1 has a differential effect on extracellular matrix production by epithelial and mesangial cells from glomeruli. TGF-β1 released in the glomerulus secondary to injury could thus affect both cell types and lead to increased intraglomerular production of proteoglycans, whereas the increased fibronectin, type IV collagen, and laminin may primarily originate from the epithelial cells.

Glomerular extracellular matrix is a complex superstructure of interacting macromolecules that surrounds the mesangial cells and, in a specialized form as the glomerular basement membrane, separates the capillary endothelium from the visceral epithelium [1]. The glomerular extracellular matrices contribute importantly to the structural organization of the glomerulus and to the biophysical properties that govern glomerular filtration and permeability [2]. Moreover, progressive glomerular disease is usually associated with marked thickening and/or expansion of the matrices [3–6].

In the primitive glomerulus endothelial cells contribute to the formation of the early glomerular basement membrane, however, with development endothelial synthesis of matrix molecules seems to stop [1]. Thus in the mature glomerulus it is likely that mesangial and epithelial cells are primarily responsible for biosynthesis and maintenance, respectively, of the mesangial matrix and the glomerular basement membrane. These two types of glomerular matrices are each composed principally of heparan sulfate proteoglycans, chondroitin/dermatan sulfate proteoglycans, fibronectin, laminin, and type IV collagen [1, 7, 8]. The biosynthesis of several of these key matrix components has recently been shown to be regulated by transforming growth factor-β (TGF-β) [9, 10].

TGF-β is a prominent member of a family of cell regulatory proteins and is unique among growth factors in its broad effects on extracellular matrix [9–11]. Three isoforms of TGF-β (1, 2 and 3) have been identified in mammals [11]. TGF-β1 stimulates synthesis of collagen, fibronectin and proteoglycans in a number of cultured cell lines [9, 10] and when injected in vivo it causes accumulation of extracellular matrix [12]. There is strong evidence for the involvement of TGF-β1 in the accumulation of extracellular matrix in kidney disease. In a rat model of experimental glomerulonephritis induced by specific immunological injury to the mesangial cell, there is a striking increase in proteoglycan and fibronectin production by isolated nephritic glomeruli in culture [13]. Moreover, conditioned medium from the nephritic glomerular cultures contained increased amounts of active TGF-β1, and there was an increased expression of TGF-β1 mRNA and TGF-β1 protein in the glomeruli. Addition of anti-TGF-β1 to the glomerular cultures markedly suppressed the synthesis of proteoglycans and fibronectin. In a related study, administration of anti-TGF-β1 to glomerulonephritic rats, markedly suppressed the histological manifestation of extracellular matrix accumulation [14].

In a study on isolated rat mesangial cells we found that TGF-β1 strongly induced the production of two chondroitin/dermatan sulfate proteoglycans, biglycan and decorin, by the cultured mesangial cells [15]. The synthesis of the other matrix components elevated in the glomerulonephritis model was not increased in the TGF-β1-treated mesangial cells. Since TGF-β1 production in the glomerulus is also likely to affect the epithelial cells, we designed the current study to characterize the action of TGF-β1 on extracellular matrix production by rat glomerular epithelial cells in culture. We found that TGF-β1, in addition to enhancing the synthesis of proteoglycans, increased the production of fibronectin and type IV collagen by the epithelial cells. This difference in the biosynthetic profile of matrix production by mesangial and epithelial cells suggests that the epithelial cells may be the cell type that contributes the nonproteoglycan component to the TGF-β1 induced expanded matrix in experimental glomerulonephritis.

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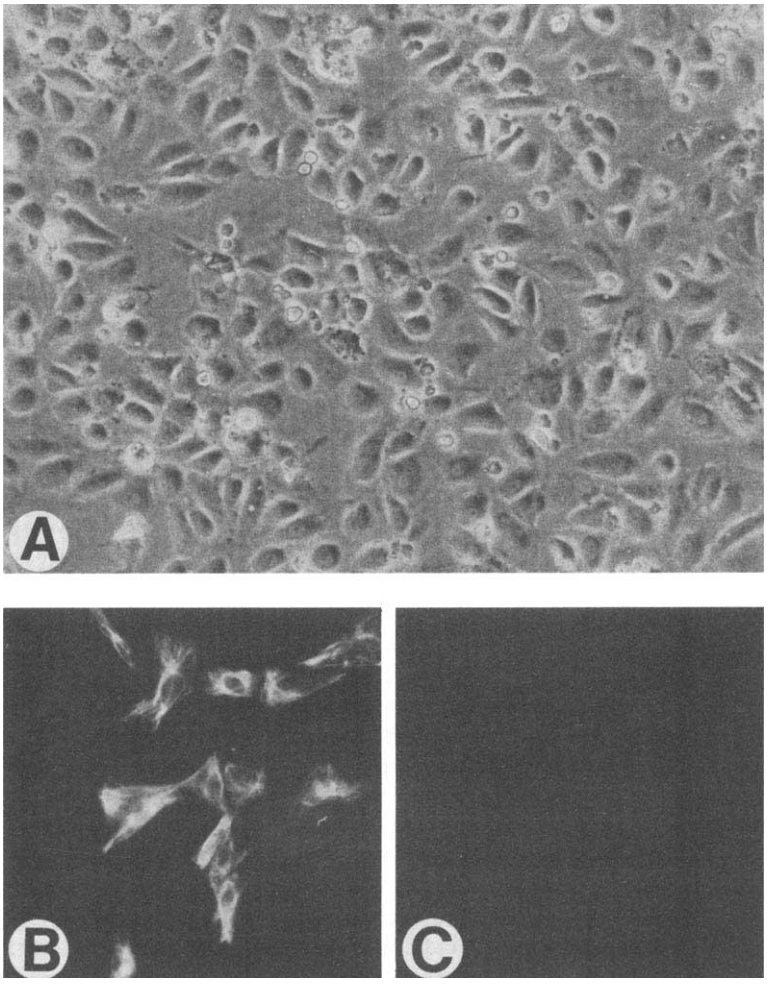

Fig. 1. *Morphological and immunofluorescence identification of cultured glomerular visceral epithelial cells.* Typical cobblestone appearance of cells grown on a laminin coated plate (**A**). Cells were uniformly stained with an antibody to vimentin (**B**) but did not stain with an antibody to cytokeratin (**C**), indicating that they are of visceral epithelial cell origin. Magnification ×500.

Methods

Growth factors and antibodies

Porcine TGF-β1 was obtained from R&D Systems, Inc. (Minneapolis, Minnesota, USA). Human platelet-derived growth factor (PDGF), and recombinant human interleukin-1 (IL-1), alpha and beta, were from Collaborative Research, Inc. (Bedford, Massachusetts, USA), recombinant human tumor necrosis factor (TNF) was from Amgen (Thousand Oaks, California, USA). Goat anti-human types I, III and IV collagen antibodies were purchased from Southern Biotechnology Associates, Inc. (Birmingham, Alabama, USA). The antibody to type IV collagen reacts with the α_1 and α_2 subunits present in the helical domains of the type IV collagen molecule contained in the glomerular basement membrane. Rabbit antibodies to mouse fibronectin, were affinity purified on fibronectin and laminin, respectively, and isolated by chromatography as described [16, 17]. Rabbit antibody to mouse type VI collagen was absorbed with normal mouse serum coupled to Sepharose and used as absorbed serum [18]. Each of these reagents specifically immunoprecipitated the appropriate antigen from medium conditioned by metabolically labeled cells [16–18 and results in this paper]. Rabbit antiserum produced against a synthetic peptide from the core protein of human biglycan was a generous gift from Dr. L. W. Fisher, National Institutes of Health. Monoclonal antibody to rat Thy-1 was obtained from Accurate Chemicals (Westbury, New York, USA) and goat anti-human factor VIII from Miles Laboratory Inc. (Kankokee, Illinois, USA). Rabbit anti-rat FX1A antibody was produced by immunization with FX1A prepared from the kidney of Sprague-Dawley rats as described [19]. FITC and alkaline phosphatase labeled anti-rabbit IgG or anti-goat IgG were obtained from Cappel (Melvern, Pennsylvania, USA).

Cell culture

Glomerular epithelial cells were obtained from outgrowth of intact glomeruli obtained from six to eight-week-old Sprague-Dawley rats. To isolate epithelial cells, intact glomeruli were placed in flasks coated with type I collagen (Vitrogen, Collagen Corp., Palo Alto, California, USA). The growth medium was RPMI 1640 (Cell-Gro, Washington, D.C., USA) supplemented with 20% heat-inactivated fetal calf serum (FCS; Hyclone, Logan, Utah, USA), 50 U/ml penicillin, 100 μg/ml streptomycin, 0.66 U/ml insulin and 300 mg/ml L-glutamine. After seven days, outgrowing cells were detached with 0.025% trypsin-

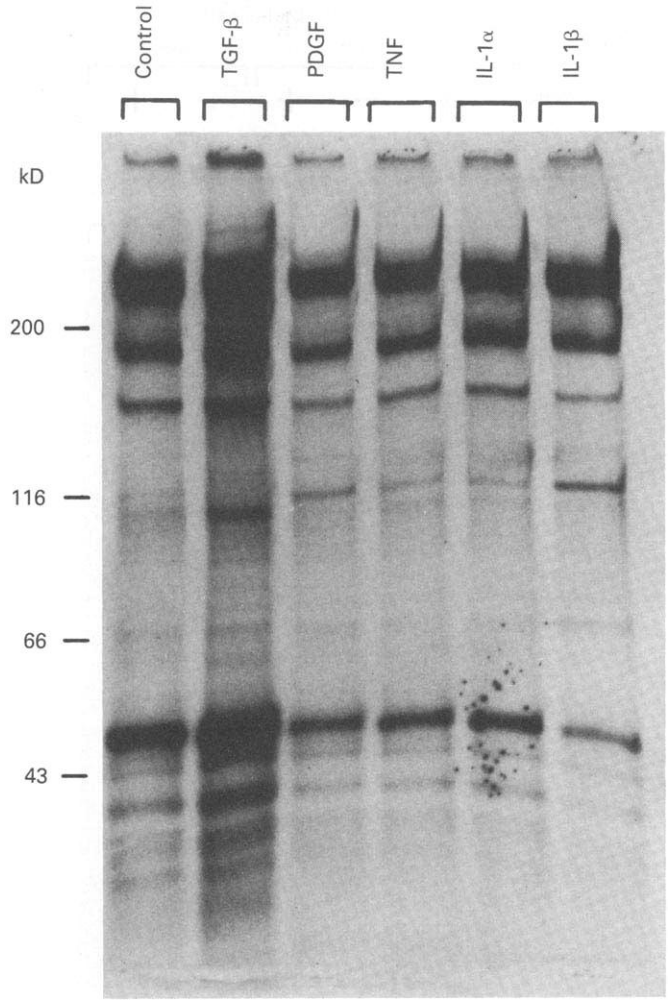

Fig. 2. *Effects of growth factors on synthesis of proteins by glomerular epithelial cells.* Cultured glomerular epithelial cells were treated with growth factors for 48 hours and metabolically labelled with ^{35}S methionine. Equal volumes of conditioned media were analyzed by SDS-PAGE and fluorography. TGF-β1 treatment increased the density of several bands derived from proteins secreted into the medium.

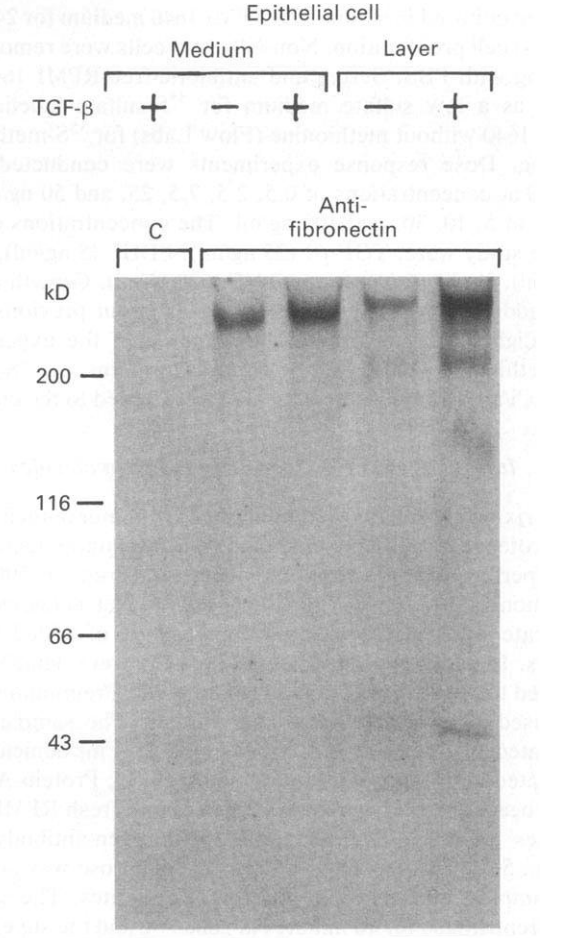

Fig. 3. *Effect of TGF-β1 on fibronectin production by glomerular epithelial cells.* Equal volumes of media and extracts of cell layers from control cells or cells treated with 25 ng/ml of TGF-β1 were immunoprecipitated with antisera to fibronectin. Preimmune serum was used as control (C). TGF-β1 increased fibronectin secretion into the medium and deposition into the cell layer.

0.5 M EDTA (Flow Labs, McLean, Virginia, USA). Glomerular debris was removed by passing the material through a 30-mesh screen. A highly enriched epithelial cell population was produced by using an indirect panning technique modified from the method of Wyoocki and Sato [20]. In this procedure mesangial cells were preferentially eliminated by binding to plates coated with anti-Thy-1 antibody; mesangial cells but not epithelial possess a Thy-1-like epitope on their surface [21]. Glomerular cells were added to 60 × 15 mm polystyrene plates, which were precoated with mouse anti-Thy-1 antibody by 12 hours incubation at 4°C. Nonadherent cells were removed and placed in 6-well plates precoated with laminin by incubating 1 ml of laminin (Collaborative Research) dissolved in phosphate buffered saline (PBS) at 10 μg/ml overnight at 4°C. The solution was removed, the plates washed ×3 with PBS and used as laminin coated plates. In separate experiments, we found no differences in cell morphology or response to TGF-β when using laminin or type I collagen plates. Laminin was arbitrarily chosen for use in the remainder of the study.

Glomerular epithelial cells were identified by: (1) characteristic polygonal morphology (Fig. 1A), (2) uniform staining with antibody to FX1A (Heymann antigen) [22], (3) sensitivity to aminonucleoside of puromycin, and (4) no staining with anti-Thy-1 or anti-Factor VIII antibody. Contamination of glomerular epithelial cells by proximal tubular cells was examined by alkaline phosphatase staining; less than 2% of the cells were alkaline phosphatase positive. To distinguish glomerular visceral from parietal epithelial cells, coverslip preparations were stained with antibodies to cytokeratin (Labsystems, Helsinki, Finland) and vimentin (BioGenex Laboratories, San Ramon, California, USA). Glomerular visceral epithelial cells are positive for vimentin and negative for cytokeratin, whereas parietal epithelial cells are negative for vimentin and positive for cytokeratin [23, 24]. Using this approach our cells were shown to be visceral epithelial cells (Fig. 1 B and C).

Biosynthetic radiolabeling

Glomerular epithelial cells were added to laminin-coated 6-well multiwell plates at a concentration of 5×10^5 cells per

well and cultured in serum-free RPMI 1640 medium for 24 hours to arrest cell proliferation. Non-adherent cells were removed by washing with PBS. Serum and antibiotic-free RPMI 1640 was added as a low sulfate medium for ^{35}S-sulfate labeling and RPMI 1640 without methionine (Flow Labs) for ^{35}S-methionine labeling. Dose response experiments were conducted using TGF-β at concentrations of 0.5, 2.5, 7.5, 25, and 50 ng/ml and PDGF at 5, 10, 30, and 100 ng/ml. The concentrations chosen for the study were: TGF-βI (25 ng/ml), PDGF (5 ng/ml), IL-1α (5 U/ml), IL-1β (5 U/ml) and TNF (500 U/ml). Growth factors were added to the media for 48 hours as in our previous study [15]. Eighteen hours prior to termination of the experiment, ^{35}S-methionine (100 μCi/ml), to label proteins or ^{35}S-sulfate (200 μCi/ml) to label proteoglycans, were added to the cultures.

Identification of extracellular matrix molecules

Matrix glycoproteins were identified by immunoprecipitation and proteoglycans by enzyme digestion. Immunoprecipitations were performed by adding 100 μl of antiserum to 500 μl of conditioned medium or 300 μl of cell extract collected from duplicate wells in the presence or absence of added growth factors. In additional duplicate wells, cells were detached and counted to ensure uniformity of cell number. Preimmune serum was used in parallel control experiments. The samples were incubated overnight at 4°C with mixing in 4 ml conical tubes precoated with bovine serum albumin (BSA). Protein-A-Sepharose beads (Sigma) were preincubated with fresh RPMI for 60 minutes at 22°C. To precipitate the antigen-antibody complexes, 50 μl of suspended protein-A-Sepharose was added to the samples, and mixed at 4°C for 120 minutes. The samples were centrifuged for 10 minutes at 2000 × g and the supernatant removed. The pellets were washed 10 times with 1 ml of ice cold PBS containing 0.5 M NaCl, 0.1% SDS and 1% Triton X-100 pH 7.4. Finally, the pellets were washed with ice cold PBS, transferred to new tubes, recentrifuged, and washed three times with PBS. The pellets were dissolved in 40 μl of SDS-PAGE sample buffer containing 3% SDS and 10% β-mercapto-ethanol (Sigma) and boiled for five minutes. To guarantee that immunoprecipitation resulted in complete recovery of the matrix components from the conditioned medium, varying concentrations of antiserum and conditioned medium were utilized. The samples were analyzed by SDS-PAGE and densitometry. These experiments showed a linear relationship between densitometric values of the gel and the amount of antisera or medium utilized. Also gels run from the supernatants from the immunoprecipitated medium showed total absence of the respective matrix component indicating complete removal of the component by the antiserum.

Enzymatic digestion to identify proteoglycans was performed on conditioned media after biosynthetic labeling. Aliquots of medium (25 μl) were mixed with 100 milliunits of chondroitinase ABC or chondroitinase AC both in 100 mM Tris-HCl, pH 7.5, 10 mM calcium acetate, 2 mg/ml BSA or 100 milliunits of heparinase II in 50 mM Tris-HCl, pH 7.4, 1 mM calcium chloride, 5 mM calcium acetate. All samples also received 1 mM PMSF, 5 mM benzamidine, 100 μg/ml soy bean trypsin inhibitor, 10 μg/ml leupeptin and 10 μg/ml antipain. All materials were obtained from Sigma. Chondroitinase-containing mixtures were incubated at 37°C for 1.5 hours, and heparinase containing mixture at 22°C for 1.5 hours. At termination samples were prepared for SDS-PAGE.

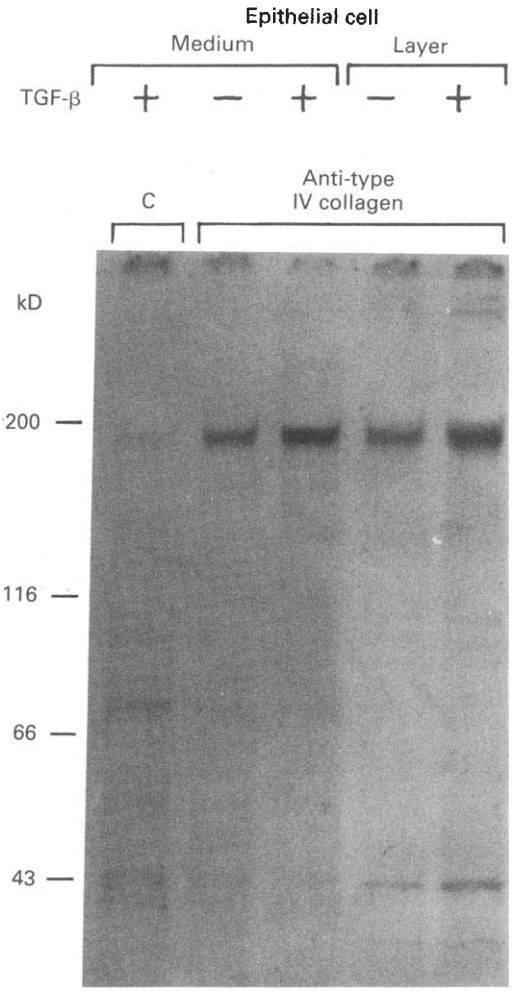

Fig. 4. *Effect of TGF-βI on type IV collagen production by glomerular epithelial cells.* Equal volumes of media and extracts of cell layers from control cells or cells treated with 25 ng/ml TGF-βI were immunoprecipitated with antisera to type IV collagen. Preimmune serum was used as control (C). TGF-βI increased type IV collagen secretion into the medium and deposition into the cell layer.

Proteoglycans were also characterized by gel filtration. ^{35}S-sulfate labeled fractions from a Sepharose CL-6B column were dialyzed against distilled water and lyophilized. Samples were dissolved in 2 ml of 0.1 M sodium acetate 0.1 M Tris, pH 7.3, containing protease inhibitors as previously described [15]. Then 0.2 ml of chondroitinase ABC solution (1.25 U/ml) was added to 1.8 ml of sample and digestion carried out for 24 hours at 37°C. Samples were then fractionated on a Sepharose CL-6B column, dialyzed against distilled water, lyophilized and treated with nitrous acid under low pH (1.0) conditions. After treatment with nitrous acid, samples were rechromatographed on the Sepharose column.

To quantitate the production of proteoglycans by cultured cells, three ml of conditioned medium were fractionated on a Sepharose CL-6B column. The column was eluted with 4 M urea, 0.15 M NaCl, 50 mM Tris HCl, pH 7.2, 0.1% (vol/vol)

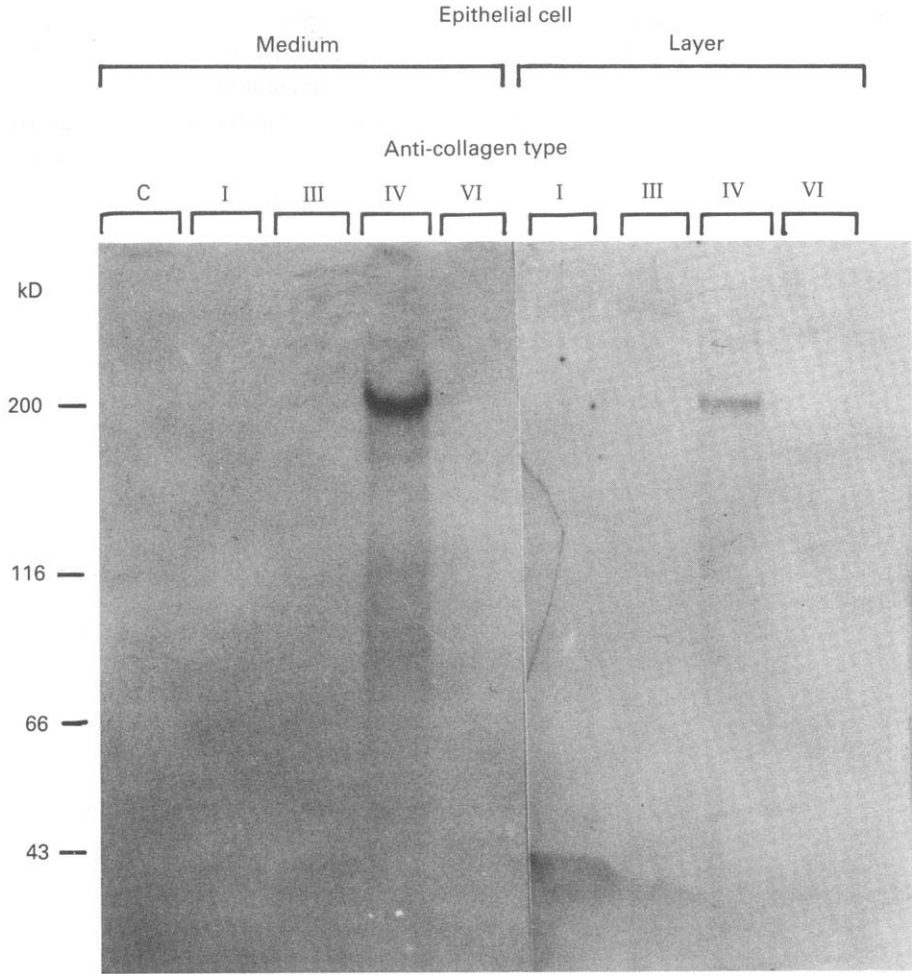

Fig. 5. *Effect of TGF-β1 on collagen production by glomerular epithelial cells.* Equal volumes of media and extracts of cell layers from cells treated with 25 ng/ml of TGF-β1 were immunoprecipitated with antisera to types I, III, IV and VI collagen. Preimmune serum was used as control (C). TGF-β1 failed to induce the production of types I, III or VI collagens.

Triton X-100. The radioactivity in each effluent fraction was determined by a Beckman LS 2800 liquid scintillation counter.

Electrophoretic technique

All samples for SDS-PAGE were mixed with sample buffer containing 3% SDS, 1 mM PMSF and 10% β mercaptoethanol and heated for five minutes at 100°C. Aliquots (20 μl) were equally applied to 4 to 12% gradient gels (Novex, Encinitas, California, USA) and run for three hours at constant power. Gels were all run reduced and were stained with Coomassie Blue. Molecular size markers were from Pharmacia (Uppsala, Sweden). Fluorography was performed by incubating gels in Enlightning (New England Nuclear, Boston, Massachusetts, USA). Typical exposure times for ^{35}S methionine were one to two days and for ^{35}S sulfate three to five days. Fluorograms were scanned with an ultrascan XL Enhanced Laser Densitometer (Pharmacia) to compare and quantitate the relative intensities and mobilities of the protein and proteoglycan bands. Fluorograms exposed for different time periods were scanned and the densitometric values were found to have a linear relationship to exposure time.

Results

Effects of TGF-β1 on production of extracellular matrix proteins

Biosynthetic labeling of cultured glomerular epithelial cells with ^{35}S-methionine was employed to label newly synthesized matrix proteins. Figure 2 shows that exposure to TGF-β1 increased the density of several protein bands identified by SDS-PAGE. In contrast, the other growth factors tested had no significant effect on the protein synthesis pattern. In a separate experiment higher doses of PDGF (10, 30 and 100 ng/ml) were tested and showed no effect on stimulating protein synthesis (data not shown). Immunoprecipitation of the conditioned media and the extracted cell layers showed that the major effects of TGF-β1 were to increase the quantity of fibronectin (Fig. 3) and type IV collagen (Fig. 4) secreted into the medium and incorporated into the matrix of the cell layer. In five independent experiments the increase in fibronectin secretion averaged 306% and varied between 250 and 370%; type IV collagen secretion averaged 329% and varied between 237 and 412%. There was no visible effect on type I, III or VI collagen (Fig. 5). TGF-β1 did not alter secretion of laminin, or a 150 kD band presumed to be entactin/nidogen. However, deposition of

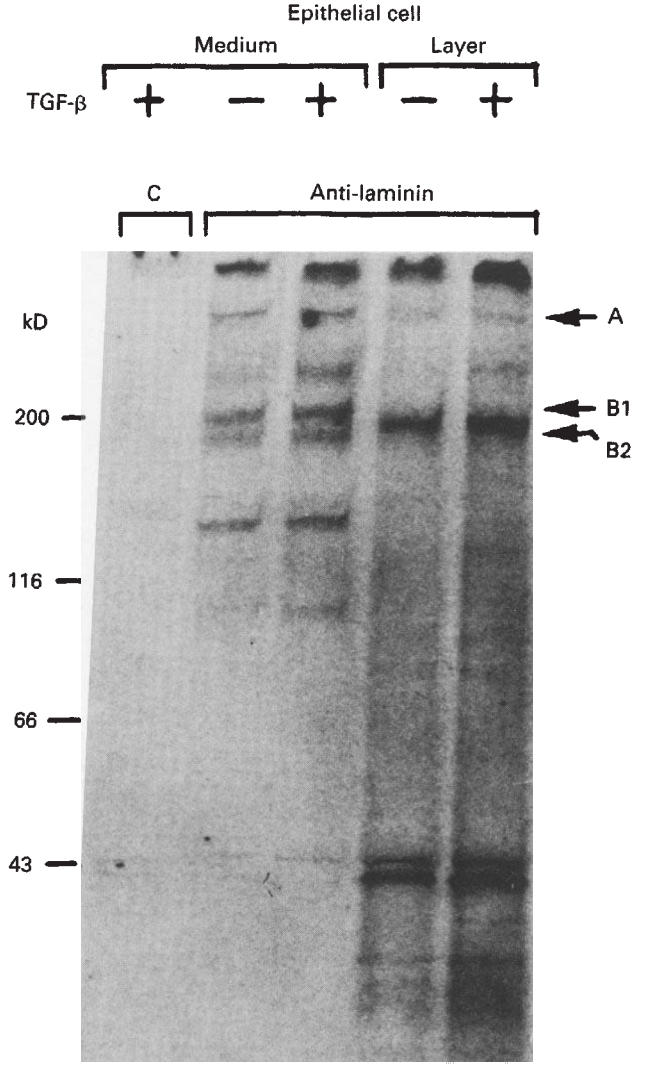

Fig. 6. *Effect of TGF-β1 on laminin production by glomerular epithelial cells.* Equal volumes of media and extracts of cell layers from control cells or cells treated with 25 ng/ml of TGF-β1 were immunoprecipitated with antisera to mouse laminin. Preimmune serum was used as control (C). TGF-β1 slightly increased laminin deposition into the cell layer. The A, B1 and B2 chains of laminin were tentatively identified and are indicated by arrows and the respective letters. The bands at 300 kD and 90 kD likely represent the two proteolytic fragments of the M chain of merosin. The M chain is related to the laminin A chain and shares the B chains with laminin and, therefore, is precipitated by the anti-laminin reagent used here [28]. The band at 150 kD is thought to represent entactin/nidogen, which binds to laminin and coprecipitates with it. The unidentified bands at 40 kD seen in all lanes are nonspecific.

Table 1. Laser densitometric analysis of proteins immunoprecipitated from media and extracted cell layers of glomerular epithelial cells

Sample	Matrix proteins			
	TGF-β1	Fibronectin	Type IV collagen	Laminin
Medium	−	0.3[a]	0.2[a]	0.1[a]
	+	0.9	0.5	0.1
Cell layer	−	0.3	0.3	0.1
	+	1.3	0.7	0.3

The cells were labeled with ^{35}S-methionine and immunoprecipitated with specific antibodies.

[a] Values are major bands at or above 200 kD shown in Figs. 3, 4 and 6 but exclude bands at the top of each lane.

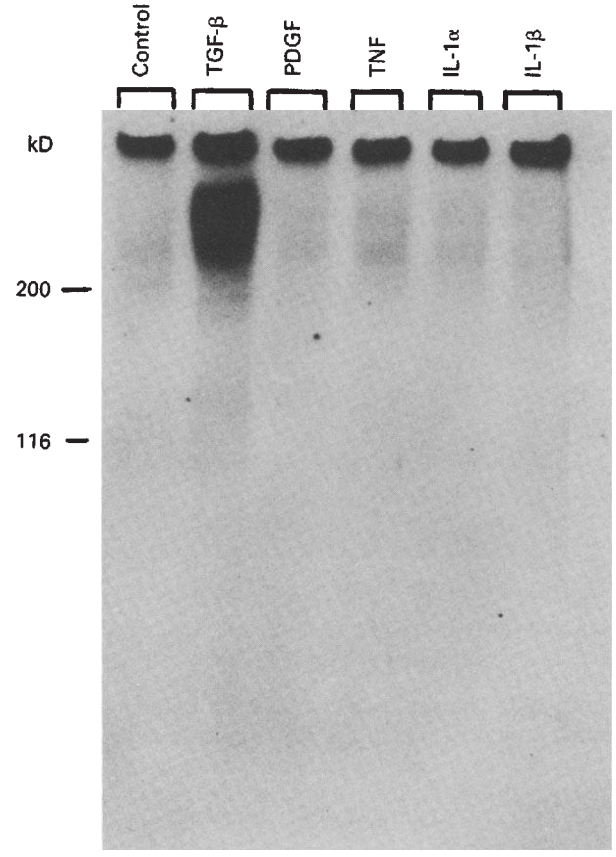

Fig. 7. *Effect of growth factors on proteoglycan production by glomerular epithelial cells.* Cultures of glomerular epithelial cells were treated with growth factors for 48 hours and metabolically labeled with ^{35}S-sulfate. Equal volumes of conditioned media were analyzed by SDS-PAGE and fluorography. TGF-β1 increased the band centered at 220 to 300 kD in conditioned media from glomerular epithelial cells. PDGF, TNF, IL-1α and IL-1β had no effect on proteoglycans.

laminin into the cell layer was slightly increased in the TGF-β1-treated cells (Fig. 6). Each of the gels shown in Figures 3, 4 and 6 was scanned by laser densitometry and the quantitative results are shown in Table 1.

Effect of TGF-β1 on production of proteoglycans

We have previously shown that TGF-β1 induces the production of two chondroitin/dermatan sulfate proteoglycans, biglycan and decorin, by rat mesangial cells [15]. For epithelial cells exposed to TGF-β1, there was a substantial increase of a ^{35}S-sulfate-labeled proteoglycan band, centered at 220 to 300 kD (Fig. 7). The increase in proteoglycan production averaged 482% and varied between 352 and 655% in five independent experiments. The other growth factors tested did not change proteoglycan production (Fig. 7), as had been observed with cultured mesangial cells [15]. Addition of increasing concentrations of TGF-β1 produced a dose response effect (Fig. 8). The maximal effect was seen at 25 ng/ml of TGF-β1, which caused

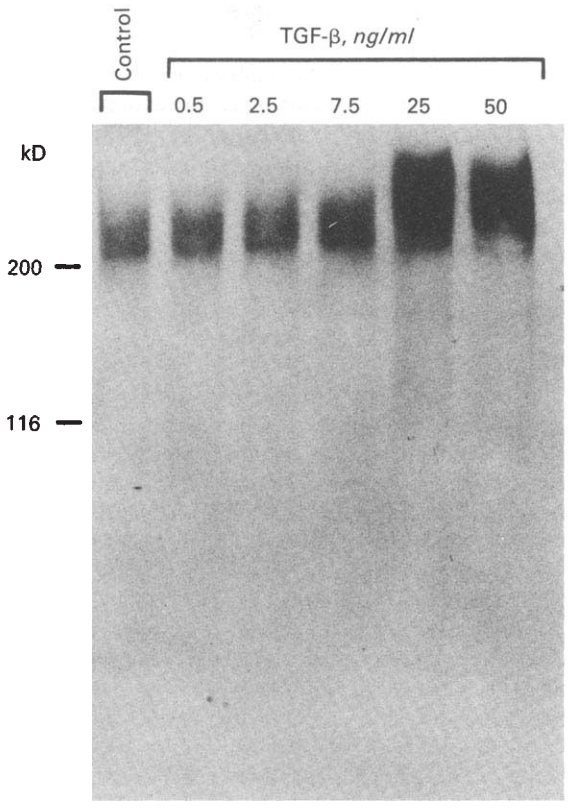

Fig. 8. *Dose response effects of TGF-β1 on proteoglycan production by glomerular epithelial cells.* Cultures of glomerular epithelial cells were treated with TGF-β1 for 48 hours and proteoglycans were analyzed as described in the legend of Figure 7. At 25 ng/ml a shift in the electrophoretic mobility and a 5-fold increase in the amount of the 220 to 300 kD proteoglycan are seen.

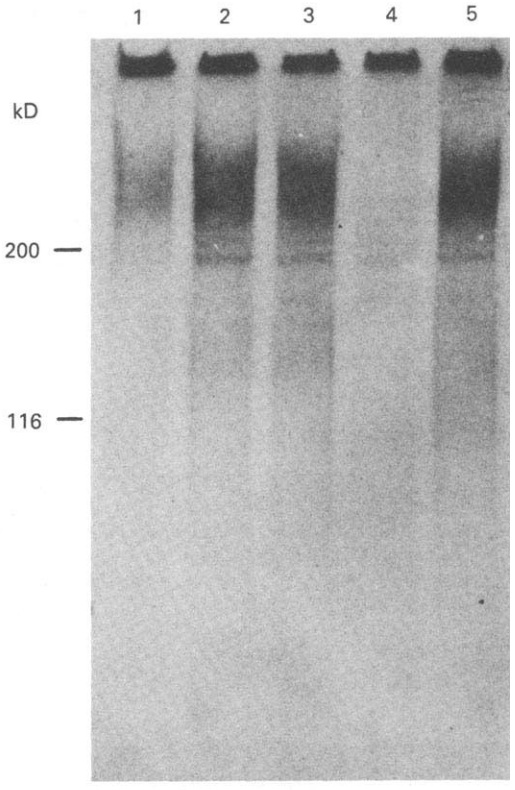

Fig. 9. *Characterization of the proteoglycans produced by glomerular epithelial cells.* Metabolically labeled medium from glomerular epithelial cell cultures treated with TGF-β1 (25 ng/ml) was subjected to specific enzyme digestion. The samples were treated with: (lane 1) saline, (2) heparinase, (3) heparitinase, (4) chondroitinase ABC, (5) chondroitinase AC. The band was digested in the sample in lane 4 indicating the presence of chondroitin/dermatan sulfate proteoglycan.

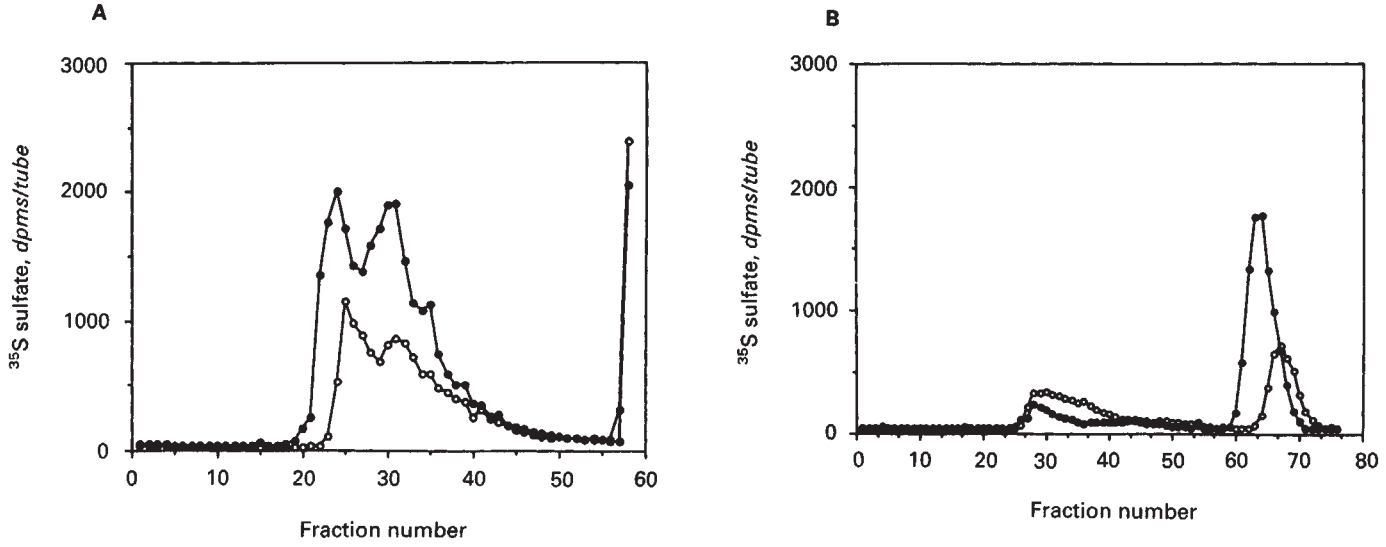

Fig. 10. *Gel filtration analysis of the proteoglycans affected by TGF-β1.* Symbols are: (—○—) TGF-β(−); (—●—) TGF-β(+). Metabolically labeled medium from control glomerular epithelial cells or TGF-β1 (25 ng/ml) treated cell cultures were applied onto a Sepharose CL-6B column before (**A**) and after (**B**) digestion with chondroitinase ABC. TGF-β1 induced a twofold increase in the production of total proteoglycans. The change was caused by an increase in the chondroitinase ABC sensitive fraction.

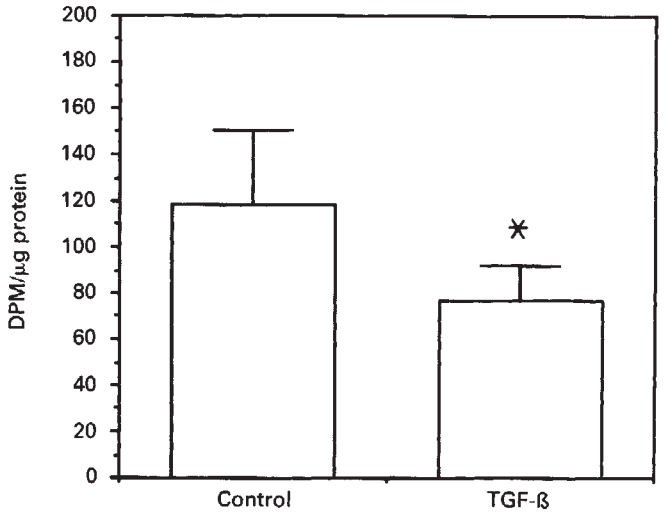

Fig. 11. *Effect of TGF-β1 on proliferation of glomerular epithelial cells.* TGF-β suppressed the ^{3}H-thymidine uptake by glomerular epithelial cells. Values are mean ± SD of six wells. $^*P < 0.001$ TGF-β compared to control analyzed by *t*-test.

a fivefold increase in proteoglycan production. In a separate dose response experiment, PDGF (10, 30 and 100 ng/ml) did not significantly affect proteoglycan synthesis (data not shown). Extraction of the cell layer showed only a slight increase in proteoglycan incorporation following exposure to TGF-β1 (data not shown), indicating that the majority of the proteoglycan induced by TGF-β1 is released into the medium.

Digestion with enzymes was used to determine the type of proteoglycans induced by TGF-β1 in the epithelial cell cultures. The proteoglycan at 220 to 300 kD was found to be degraded by chondroitinase ABC, but not by heparinase, heparitinase or chondroitinase AC (Fig. 9); this indicates that it is a chondroitin/dermatan sulfate proteoglycan. The proteoglycan was further characterized as being biglycan by immunoprecipitation using antiserum to a synthetic peptide of the core protein of human biglycan (data not shown). A proteoglycan band, too large to enter the gel, is seen at the top of each lane in Figure 9. In order to include this band in our analysis, we evaluated the ^{35}S-sulfate labeled proteoglycans by a combination of digestion with specific enzymes and gel filtration chromatography. In control experiments we found that epithelial cells secrete proteoglycans that elute as two broad peaks; each of these peaks is significantly increased following addition of TGF-β1 (Fig. 10A). Following digestion with chondroitinase ABC, 60% of the radioactivity was removed from the control conditioned medium, whereas, 85% disappeared from the conditioned medium from cells exposed to TGF-β1 (Fig. 10B). Nitrous acid specifically digests heparan sulfate [25]. When samples containing these peaks remaining after chondroitinase ABC digestion were treated with nitrous acid, the peaks disappeared and the radioactivity was found in the void volume (data not shown). These results were confirmed in three separate experiments and indicate that the predominant effect of TGF-β1 on epithelial cell proteoglycan production is on the synthesis of chondroitin/dermatan sulfate proteoglycans, with little effect on the production of heparan sulfate proteoglycans.

Effect of TGF-β on cell proliferation

An increase in extracellular matrix production by cultured cell exposed to a growth factor, may be secondary to stimulation of cell proliferation. Examination of ^{3}H-thymidine incorporation as a measure of DNA synthesis and proliferation showed that TGF-β added to subconfluent cultures of glomerular epithelial cells reduced ^{3}H-thymidine incorporation by an average of 30% in three experiments (Fig. 11).

Discussion

The results of our study show that, among various growth factors, TGF-β1 alone increased extracellular matrix production by cultured glomerular epithelial cells. Our results also indicate a difference in the response to TGF-β1 between glomerular epithelial and mesangial cells. TGF-β1 strikingly increased the production of fibronectin, type IV collagen and a chondroitin/dermatan sulfate proteoglycan, biglycan, by epithelial cells. In contrast, as demonstrated previously, the predominant action of TGF-β1 on mesangial cells was restricted to two chondroitin/dermatan sulfate proteoglycans, biglycan and decorin [15].

TGF-β1 substantially increased the synthesis by glomerular epithelial cells of both fibronectin, a glycoprotein found in connective tissue matrices [26] and type IV collagen, a major basement membrane protein [1, 7]. A lesser effect was seen on another key basement membrane glycoprotein, laminin. In an earlier study it was shown that TGF-β1 stimulated fibronectin synthesis but did not affect the production of collagenase-sensitive material by murine glomerular epithelial cells [27]. The fact that we found a clear increase in type IV collagen synthesis may be due to the fact that we employed a different species, and utilized immunoprecipitation to identify specific collagen molecules.

TGF-β1, and probably TGF-β2 and 3 are unique among growth factors in the extent to which their multiple actions are mediated through regulation of extracellular matrix synthesis [11]. The glomerular epithelial cell can now be added to the list of cell lines that have been shown to increase production of matrix components following exposure to TGF-β1. The current results also help illuminate our previous finding of increased production of matrix components by cultured nephritic glomeruli [13]. We have shown that antibody induced injury to the mesangial cell in vivo results in expression of TGF-β1 mRNA and TGF-β1 protein in the nephritic glomeruli. Following injury, cultured nephritic glomeruli were found to have increased production of proteoglycans and fibronectin which could be blocked by addition of anti-TGF-β1. Although the experimental injury was confined to the mesangial cell, it is likely that the newly released TGF-β1 induced matrix production by both the mesangial cell and the glomerular epithelial cell. Although both cell types could have contributed to the increased proteoglycan production, the pattern of TGF-β1 stimulated matrix production revealed by the present study suggests that the epithelial cells contribute to the increased fibronectin, and other nonproteoglycan components, synthesized by nephritic glomeruli and deposited into the expanded mesangial matrix in experimental glomerulonephritis.

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